

Insulin/IGF-I-signaling pathway: an evolutionarily conserved mechanism of longevity from yeast to humans

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Barbieri, Michelangelo, Massimiliano Bonafè, Claudio Franceschi, and Giuseppe Paolisso. Insulin/IGF-I-signaling pathway: an evolutionarily conserved mechanism of longevity from yeast to humans. *Am J Physiol Endocrinol Metab* 285: E1064–E1071, 2003; 10.1152/ajpendo.00296.2003.—Although the underlying mechanisms of longevity are not fully understood, it is known that mutation in genes that share similarities with those in humans involved in the insulin/insulin-like growth factor I (IGF-I) signal response pathway can significantly extend life span in diverse species, including yeast, worms, fruit flies, and rodents. Intriguingly, the long-lived mutants, ranging from yeast to mice, share some important phenotypic characteristics, including reduced insulin signaling, enhanced sensitivity to insulin, and reduced IGF-I plasma levels. Such genetic homologies and phenotypic similarities between insulin/IGF-I pathway mutants raise the possibility that the fundamental mechanism of aging may be evolutionarily conserved from yeast to mammals. Very recent findings also provide novel and intriguing evidence for the involvement of insulin and IGF-I in the control of aging and longevity in humans. In this study, we focus on how the insulin/IGF-I pathway controls yeast, nematode, fruit fly, and rodent life spans and how it is related to the aging process in humans to outline the prospect of a unifying mechanism in the genetics of longevity.

aging; longevity; insulin-like growth factor I

THE POTENTIAL LINK between aging and insulin/insulin-like growth factor I (IGF-I) signaling has attracted substantial attention on the basis of several pieces of evidence showing that disruption of the insulin/IGF-I-signaling cascade can significantly extend life span in diverse species, including yeast, worms, fruit flies, and rodents. Although the underlying mechanisms of longevity are not fully understood, it is known that mutation in genes that share similarities with those of humans involved in the insulin/IGF-I signal response pathway can significantly extend life span (5, 12, 25, 66). Examples include *daf-2*, *age-1*, and *daf-16* mutants of the nematode *Caenorhabditis elegans*, yeast *sch9*, and *CYR*-null mutants, *InR* and *Chico* homozygous mutant female flies, and long-lived *Prop1*- and *Pit1*-mutant mice (5, 12, 25, 66).

Intriguingly, long-lived mutants, ranging from yeast to mice, share some important phenotypic characteristics, including reduced insulin signaling, enhanced sensitivity to insulin, and reduced IGF-I plasma levels, with reduced oxidative damage of macromolecules and increased stress resistance being the likely final common pathway of these effects. Such genetic homologies and phenotypic similarities of insulin/IGF-I pathway mutants raise the possibility that the fundamental mechanism of aging may be evolutionarily conserved from yeast to mammals (8, 42).

Interestingly, in the recent past, a large amount of research has focused attention on invertebrates and laboratory animal models. Indeed, very recent findings also provide novel and intriguing evidence for the involvement of insulin and IGF-I in the control of aging and longevity in humans. In particular, it has been recently demonstrated that polymorphic variants of IGF-I receptor (IGF-IR) and phosphatidylinositol 3-kinase (PI3K) genes affect IGF-I plasma levels and human longevity (8). That study represents the first indication that genetic variability at the insulin/IGF-I-signaling response pathway plays a role in human longevity, indicating that the impact of these genes on species longevity is an evolutionarily conserved property (Fig. 1).

Thus we will review all previous evidence for the involvement of the insulin/IGF-I pathway in the control of aging and longevity. In particular, we will focus on how the insulin/IGF-I pathway is regulated, how it controls nematode, fruit fly, and rodent aging and life span, and how it is related to the aging process in humans to outline the prospect of a unifying mechanism in the genetics of longevity.

C. ELEGANS

The important involvement of the insulin/IGF-I-signaling pathway in the evolutionarily conserved mechanisms that control aging and life span was first suggested by results obtained in invertebrates (15, 72).

In *C. elegans*, an insulin-like signaling cascade, consisting of proteins encoded by the genes *daf-2*, *age-1*,

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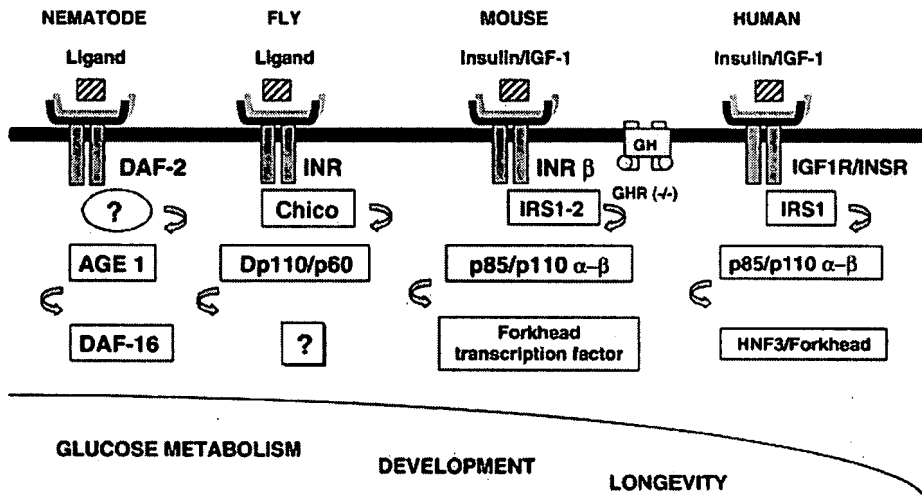


Fig. 1. Insulin/IGF-I signaling pathway: human homologies with nematodes, fruit flies, and mice.

akt-1, *akt-2*, *daf-16*, and *daf-18*, regulates dauer diapause and reproduction and has considerable influence on adult life span. The products of these genes revealed insulin/IGF-I signal transduction as a central regulator of aging (71, 73). The gene *daf-2* encodes an insulin/IGF-I receptor-like protein and may be the common ancestor of human insulin receptor, human IGF-I receptor (IGF-IR), and the human insulin receptor-related receptor, because its sequence is equally distant from all of them (71, 73).

Animals with weak *daf-2* mutations age more slowly than wild-type animals and live much longer. Furthermore, mutation of the downstream *age-1* gene, which encodes a protein similar to the mammalian p110 catalytic subunit of PI3K, leads to a 65% increase in mean life span (36, 48). These effects have been shown to depend on the integrity of the dauer formation protein (DAF)-16, which has similarity to a family of mammalian forkhead transcription factors (40, 51). *daf-16* appears to play a unique role in life span regulation and encodes a member of the hepatocyte nuclear factor-3 (forkhead family of transcriptional regulators) (27). A null mutation in *daf-16* suppresses the phenotype of *daf-2* and *age-1*. The eventual activation of the transcription factor indicates that the insulin/IGF-I receptor-like signal pathway regulates aging by modulating gene expression (40, 51).

Interestingly, not only is *daf-2* related to the mammalian insulin receptor by sequence homology but it also regulates metabolism: decreased DAF-2 signaling has been shown to induce important metabolic changes (36, 73). In particular, null mutations in *daf-2* or *age-1* cause constitutive arrest at the dauer larval stage (36, 43). Dauer larvae have slowed metabolic rates, store large amounts of fat, and live longer than reproductive adults. Furthermore, worms with mutation of the *daf-2* pathway exhibit increased resistance to oxidative stress: *daf-2* mutants express high levels of antioxidant enzymes such as catalase and superoxide dismutase (SOD), and the *age-1* mutation partially prevents the age-associated decrease of catalase in adults of *C. elegans* (29, 30, 78).

The lower level of free radicals in *daf-2* insulin-like signaling mutants has been shown to be essential for life span extension (50). Indeed, the gene *ctl-1*, which encodes a cytosolic catalase, is required for the extension of adult life span by *daf-2* (68). These findings highlight the central position of oxidative stress in the aging-regulatory machinery.

Because the *C. elegans* genome contains 37 insulin-like ligands that are expressed mainly in neurons but are also found in intestine, muscle, epidermis, and gonad (23, 58), the site of action of insulin/IGF-I signaling has been further investigated. Interestingly, the same signals from different tissues do not influence aging equally. In this regard, recent studies have demonstrated that DAF-2 signaling in the nervous system is a critical regulator of longevity (78). Restoring the *daf-2* pathway in neurons, but not in muscle or intestine, reduced the life span of *daf-2* mutant animals to normal (78). Although the insulin/IGF-I receptor-like signal also regulates metabolism, restoration of metabolic activity in muscle and intestine by expressing *daf-2* in these tissues does not change the aging process. Indeed, *daf-2* signaling in germline and somatic gonads had a significant influence on life span as well (57).

SACCHAROMYCES CEREVISIAE

Saccharomyces cerevisiae is an attractive model for studying how glucose and energy metabolism are linked to aging. In fact, age-associated alterations in energy metabolism can be analyzed more readily in a unicellular eukaryote than in a multicellular organism with diverse, specialized cell types.

Although yeast do not have an insulin-signaling pathway, they appear to have precursors of such a metabolic control pathway that function in the glucose/nutrient-signaling cascade and are homologous to the serine/threonine kinase Akt/PKB of insulin-signaling pathways in *C. elegans* and mammals.

Interestingly, yeast, like metazoans, change their patterns of growth and physiology in response to their

environment (70). When glucose levels are high, cells upregulate glycolysis, growth, and proliferation. As glucose levels fall, the cells begin to divide more slowly and subsequently enter a stationary phase.

Moreover, genetic studies suggest that changes in glucose metabolism affect the replicative life span of *S. cerevisiae*. In particular, increased longevity occurs with mutations affecting the glucose-responsive cAMP-dependent protein kinase A (PKA) pathway, hexokinase (catalyzing the first step in the glycolytic pathway), or the Snf1 pathway (Snf1p) (3, 41). Snf1p is a serine/threonine kinase required for the normal cellular response to glucose starvation and represents the yeast homolog of AMP kinase (AMPK), a mammalian cellular "fuel gauge" (26). It is incorporated into a complex that contains Snf4p, Sip1p, Sip2p, and Gal83p. When there is limited glucose, Snf4p activates Snf1p kinase, which phosphorylates a number of target proteins including transcriptional regulators of genes involved in alternative carbon source utilization, gluconeogenesis, and respiration (26). Loss of the Snf1p activator Snf4p produces a 20% increase in life span, whereas forced expression of *SNF1* produces rapid aging (3). The mammalian homolog of Snf1p is AMPK. The AMPK pathway is activated in response to various stresses, including glucose deprivation, heat shock, hypoxia, and exercise (26). Once activated, AMPK phosphorylates some of the same downstream targets that are recognized by Snf1p, including acetyl-CoA carboxylase and glycogen synthase (26).

In yeast, the response to glucose is also mediated by two protein kinases, PKA and Sch9. Interestingly, Thevelein and de Winder (70), screening for long-lived mutants in nondividing *S. cerevisiae* yeast, demonstrated that mutation in the *CYR1/PKA* and *SCH9* genes can extend the longevity of nondividing cells up to threefold. The *CYR1* gene encodes for adenylate cyclase, which stimulates the cAMP-dependent protein kinase (PKA) activity required for cell cycle progression and growth. Indeed, the COOH-terminal region of Sch9 is highly homologous to the AGC family of serine/threonine kinases, which includes Akt/PKB. SCH9 is homologous to the serine/threonine kinase Akt/PKB of insulin-signaling pathways in *C. elegans*, *Drosophila*, and mammals. Both *Sch9* and *Cyr1* function in pathways that mediate glucose-dependent signaling, stimulate growth and glycolysis, and decrease stress resistance, glycogen accumulation, and gluconeogenesis (21, 70).

In yeast, the downregulation of glucose signaling by *cyr1* and *sch9* increases resistance to thermal stress, in part, by activating transcription factors Msn2 and Msn4, which induce the expression of genes encoding for several heat shock proteins, catalase (*CTT1*), and the DNA damage-inducible genes *DDR2* (44) and *SOD2*. MnSOD also appears to be regulated in a similar manner. Interestingly, the expression of mitochondrial *SOD2* is required for the longevity extension caused by mutations decreasing the activity of the Ras/Cyr1/PKA and Sch9 pathways. Such data confirm

that superoxide toxicity plays an important role in yeast aging and death (20, 70).

DROSOPHILA MELANOGASTER

In the fruit fly *Drosophila melanogaster*, the insulin/IGF receptor INR, the insulin receptor substrate CHICO, the PI3K Dp110/p60, and the PI3K target PKB (also known as Akt1) form a signaling pathway that regulates growth, size (77), and life span. Interestingly, the *D. melanogaster* gene insulin-like receptor (*InR*) is homologous to mammalian insulin and IGF-I receptors (4, 60) as well as *C. elegans daf-2*. Tatar et al. (67) reported that mutation of the *InR* in the fruit fly *D. melanogaster* can significantly extend adult longevity. In particular, it has been shown that females with a heteroallelic genotype *InR^{p5545}/InR^{E19}* are small and infertile and live 85% longer than do wild-type controls. The long-lived mutant flies share some important characteristics with wild-type adults that are in reproductive diapause, including increased triglycerides and SOD (a free radical scavenger) and reduced synthesis of juvenile hormone, a neurohormone that influences reproduction in insects and exhibits some functional homology to vertebrate thyroid hormones.

The life span of female *D. melanogaster* is also extended by mutation of the insulin receptor substrate (IRS) homolog *chico* (12). In this regard, Clancy et al. (12) report that null mutation of the *chico* gene that encodes IRS increases the life span of homozygous *chico¹/chico¹* female fruit flies by 48%. Homozygous males are short lived, whereas heterozygous animals of both sexes have increased longevity ($\leq 36\%$ in females and 13% in males). Indeed, flies homozygous for *chico* have increased levels of SOD, reduced body size, and greatly reduced fecundity. Experiments involving genetic rescue of the effects of *chico* on somatic growth in transgenic flies and crosses with flies heterozygous for a dominant mutation causing female sterility provided evidence that neither dwarf phenotype nor infertility is required for increased longevity of *chico* flies (12).

RODENTS

A decrease in insulin/IGF-I signaling has also been shown to extend longevity in mice. Analysis of various transgenic and knockout rodent models, characterized by shortening or extension of the life span, gives the unique possibility to evaluate the relationship between the insulin/IGF-I-signaling pathway and longevity (9, 65). At present, there are four examples of single gene mutations that extend longevity in mammals: Ames and Snell Dwarf mutant mice, *p66^{-/-}* knockout mice, and aMUPA MGMT-transgenic mice. By far, the two most widely used models are the Snell and Ames Dwarf mice (11, 65). These recessive mutant mice are homozygous for mutation at the pituitary-1 (*pit1*) (37), *Pit-1*, or *Prop-1* (18) locus, respectively, which encodes for transcription factors controlling pituitary development. Both Snell and Ames Dwarf mice demonstrate increased longevity (50% in males and 64% in females)

relative to their wild-type controls (9, 24), which generally has been attributed to growth hormone (GH)/IGF-I deficiency. Mice homozygous for such a mutation are deficient in serum GH, thyroid-stimulating hormone (TSH), and prolactin as well as IGF-I, which is secreted by liver cells upon stimulation with GH. Because of the complexities inherent in the multiple endocrine deficiencies, to assess the specific contribution of IGF-I deficiency to the observed increase in life span, studies of GH receptor-binding protein (GHRBP) and IGF-IR knockout mice have been performed (10, 14, 49). In particular, such studies demonstrated that dwarf mice with high plasma GH but a 90% lower IGF-I [GHRBP-null mice (49, 65)] live longer than the wild-type mice (14), thus suggesting that the reduction in plasma IGF-I is responsible for a major portion of the life span increase in dwarf, GH-deficient, and GHRBP-null mice. Powerful evidence for the direct role of IGF-I signaling in the control of mammalian aging was also provided by mutants for the IGF-IR *Igf1r*: loss of a single copy of the *Igf1r* gene (encoding the IGF-IR) results in a 26% increase in mouse life span (28). These mutants exhibit minimal reduction in growth with no alteration in the age of sexual maturation, fertility, metabolism, food intake, or temperature. Interestingly, life extension was associated with increased tolerance to oxidative stress and reduced phosphorylation of Shc, a gene previously implicated in the control of longevity and stress resistance in Ref. 46.

Putative mechanisms linking reduced IGF-I signaling with delayed aging include reduced insulin release combined with enhanced insulin sensitivity. Very strong support for the role of insulin signaling in the control of mammalian aging and for the involvement of this pathway in extending the life span of IGF-I-deficient mice was also provided by recent studies of Hsieh et al. (31). On the basis of results obtained in GH-, prolactin-, and TSH-deficient Snell Dwarf (*Pit1^{dw}*) mice, these authors proposed that insulin signaling via InR, IRS-1 or IRS-2, and PI3K affects genes that control life span (31). In the Snell dwarf mouse, GH deficiency leads to reduced insulin release and alterations in insulin signaling, including a decreased IRS-2 pool level, a reduction in PI3K activity and its association with IRS-2, and decreased docking of p85 α to IRS-2 and preferential docking of IRS-2 to p85 α -p110 α . These alterations would establish a physiological homeostasis that favors longevity (31). The data are consistent with the hypothesis that the decreased circulating insulin levels resulting from the *Pit1* mutation mimic a physiological state similar to that proposed to occur in the long-lived *C. elegans daf-2* mutant.

Mouse longevity is also increased by fat-specific disruption of the insulin receptor gene FIRKO (6). These animals have reduced fat mass and are protected against age-related obesity and its subsequent metabolic abnormalities, although their food intake is normal. Both male and female FIRKO mice were found to have an increase in mean life span of ~134 days (18%), with parallel increases in median and maximum life spans. Multiple intriguing changes in adipocytes un-

derlie these effects, including elevated plasma leptin relative to adipose tissue mass, reduced lipolysis, and polarization of adipocytes into populations with altered expression of fatty acid synthase (7). Thus insulin in adipose tissue may affect aging through its impact on neural targeted hormones as well as through regulation of intermediary metabolism.

Among different insulin signaling cascade components, a role of the glucose transporter GLUT4 in mouse longevity has also been demonstrated (34). Insulin-sensitive GLUT4 is the most abundant facilitative glucose transporter in muscle and adipose tissue, the major sites for postprandial glucose disposal. Interestingly, Katz et al. (34) demonstrated that functional GLUT4 protein is absolutely essential for sustained growth, normal cellular glucose and fat metabolism, and longevity. Mice deficient in GLUT4 (GLUT4-null) are growth retarded and exhibit decreased longevity associated with cardiac hypertrophy and severely reduced adipose tissue deposits (34).

A strong link between insulin/IGF-I signaling and life span in animal models comes from the literature on dietary restriction and aging (61); reducing the food (calorie) intakes of rats and mice can increase their life spans by 40% or more (76). A highly reproducible finding in such dietary restriction studies is that animals (rodents and primates) maintained on dietary restriction feeding regimens exhibit increased insulin sensitivity and enhanced glucose tolerance (33, 74). A recent study also indicates that lower levels of IGF-I could contribute to the protective effect of moderate caloric restriction against age-related pathology. Dunn et al. (19) demonstrated that calorically restricted rats have low levels of IGF-I and are resistant to *p-cristine*-induced bladder cancer. Furthermore, the replacement of IGF-I restored the incidence of bladder cancer to that of ad libitum-fed animals (19).

HUMANS

Novel and intriguing evidence for the involvement of the insulin/IGF-I cascade in the control of aging and longevity in humans also has been recently provided.

In humans, insulin sensitivity normally declines during aging, and insulin resistance is an important risk factor (22, 39) associated with a variety of intermediate phenotypes (hypertension, atherosclerosis, obesity) strongly affecting morbidity, disability, and mortality among the elderly (17, 59).

Interestingly, one of the very striking physiological characteristics recently identified in centenarians is their greatly enhanced sensitivity to insulin compared with less aged subjects (1, 53, 55). In particular, a previous study in an extremely limited number of centenarians living in southern Italy showed this group to have a preserved glucose tolerance and insulin action and lower plasma IGF-I levels compared with aged subjects (52, 55). More recently, data from 466 healthy subjects with a wide age range (range 28–110 yr) demonstrated a significant reduction of insulin resistance in subjects 90–100 yr old (54). These data sug-

gest an intriguing peculiarity of this age category and indicate that an efficient insulin response has an impact on human longevity. Moreover, the biological improvement observed in centenarians fits well with the shape of some allele frequency trajectories obtained by genetic-demographic models applied to human longevity studies (69, 79). Such trajectories indicate that, during aging, peculiar forces are likely to select people with special characteristics, allowing these individuals to reach the extreme limits of human life span. These results point out the possibility that, in humans, insulin sensitivity is a phenotype under strong selective forces during aging and is likely to represent factors that allow some individuals to reach the extreme limits of human life span.

With regard to the specific genetic background involved and on the basis of the literature mentioned above, the role of genetic variability at human loci that share similarities with the genes that regulate the insulin/IGF-I response in *C. elegans*, in *D. melanogaster*, and in mice has been recently evaluated (8). In particular, polymorphisms at *IGF-IR* (34% protein sequence identity with *C. elegans DAF-2* gene), *PI3KCB* (27% protein sequence identity with *C. elegans AGE-1* gene), *IRS-1* (30% protein sequence identity with *D. melanogaster CHICO* gene), *FOXO1A* (49% protein sequence identity with *C. elegans DAF-16* gene) have been investigated. Interestingly, the polymorphism A/G at position 3174 (codon 1013) of the *IGF-IR* gene (chromosome 15q25-q26) has been found to be associated with different IGF-I plasma levels and to be differentially represented in long-lived vs. adult subjects (8). In particular, individuals bearing at least one allele A at the *IGF-IR* locus (*IGF-IR* A⁺) have lower plasma IGF-I levels than the other subjects as well as *IGF-IR* A⁺ subjects being found at an increased proportion in long-lived individuals. Furthermore, genotype interaction of an A allele at the *IGF-IR* locus and a T allele at the *PI3KCB* locus (A⁺/T⁺ subjects) has also been shown to affect longevity and IGF-I plasma levels, with A⁺/T⁺ individuals having the lowest free IGF-I plasma levels (8). Moreover, an increased frequency of A⁺/T⁺ genotype among long-lived individuals has also been found.

The findings of these studies provide novel and intriguing evidence for the involvement of the insulin/IGF-I pathway in the control of aging and longevity in humans, indicating that the impact of these genes on species longevity is an evolutionary conserved property (Fig. 1). Indeed, the functional effects of the structural variation of the *IGF-IR* due to gene polymorphism remain unclear, and the significance of plasma IGF-I remains to be established.

IGF-I is a potent anabolic hormone that increases cellular metabolism, enhances the function of numerous tissues, and participates in glucose homeostasis (13). Furthermore, GH and IGF-I administration slows down the negative role of age on several tissues (2, 16, 62). Such a beneficial effect on tissue function, particularly evident during development (when GH and IGF-I levels are high), appears to contradict the afore-

mentioned results showing that reduced IGF-I plasma levels are associated with longevity. Indeed, previous studies demonstrated that continuously high levels of IGF-I over the life span would lead to deleterious consequences and contribute to pathological changes associated with age through the effect on cell division and/or metabolism. In fact, due to the effect of IGF-I on cellular replication, this hormone has also been linked to the development of several diseases such as cancer (32, 75). Thus it could be hypothesized that the decrease in plasma IGF-I observed in long-lived subjects might minimize the generalized mitogenic stimulus to tissues and then contribute to the reduction of age-related pathologies. Such a hypothesis fits with a recently described theory on the evolution of aging, which suggests that the expression of particular genes is beneficial early in life but becomes detrimental with age (37).

The aforesaid notwithstanding, the local expression (bioavailability) of IGF-I remains an important factor contributing to the maintenance of normal tissue function (5, 13). Interestingly, Sonntag et al. (62) hypothesized a different regulation of tissue and plasma IGF-I and speculated that the paracrine expression of IGF-I into old age may be an important factor for improving the function of individual tissues and organs in response to specific stimuli, whereas the reduction in plasma IGF-I may be sufficient to diminish a generalized mitogenic stimulus and thus influence the initiation and progression of age-related pathologies (62).

Interestingly, several findings in *C. elegans*, *Drosophila*, and mice are consistent with the hypothesis that mutations in genes of the insulin-like signaling network confer oxidative stress resistance as well as life span extension, thus suggesting that diminished oxidative damage of macromolecules might be the final common pathway of the effects of the insulin/IGF-I-signaling mutation on longevity. Several studies in humans have shown free radicals to play a key role in the pathogenesis of a wide variety of clinical diseases, including cardiovascular diseases, atherosclerosis, and diabetes mellitus (63, 64). Furthermore, healthy centenarians, the best living model of longevity, have been shown to have a low degree of oxidative stress and high antioxidant defenses, which seem to be important in guaranteeing their extreme longevity (45, 47, 56).

These results suggest that the genetic link among insulin-like signaling, oxidative stress, and longevity, originally discovered in nonvertebrates and mammals, also exists in humans. Unfortunately, information on the molecular mechanism defining the interrelation among the insulin/IGF-I-signaling pathway with oxidative stress resistance and longevity is lacking.

CONCLUSIONS

From the data presented, similarities between insulin/IGF-I-regulatory systems in yeast, worms, flies, mammals, and humans emerge: 1) the systems are composed, at least in part, of homologous genes and pathways; 2) each regulates a similar set of processes:

oxidative stress resistance, metabolism regulation, food utilization pathways, and life span; and 3) each serves a similar biological function, allowing animals to postpone reproduction during unfavorable environmental conditions.

Such similarities are striking and suggest that the insulin/IGF-I-regulatory system arose early in evolution and that the fundamental mechanism of aging may be evolutionarily conserved from yeast to mammals, not excluding humans.

Naturally, caution must be exercised in interpreting these novel and intriguing findings. In fact, although similar phenotypic characteristics have been identified, physiological differences among invertebrates and mammals should be taken into account.

For example, control of aging by the *daf-2* pathway in *C. elegans*, or by the gene insulin-like receptor in *D. melanogaster*, involves diapause (i.e., an inactive, non-feeding dauer stage of the life cycle and reproductive diapause, respectively) that has no obvious counterpart in most mammals or humans. Furthermore, whereas IGF-I signaling in long-lived mutant and knockout mice is similar to the finding of reduced insulin/IGF-I signaling in long-lived mutant invertebrates, IRS expression does not occur in *chico* flies but is increased in *Prop1^{df}* mice. In humans, insulin receptor mutations cause diabetes rather than longevity. Such differences strongly suggest that, although it is admitted that the fundamental mechanism of aging/longevity is evolutionarily conserved, differences in insulin/IGF-I-signaling physiology have occurred during evolution.

In particular, insulin/IGF-I pathways have increased greatly in complexity during evolution (35). Worm and fly have only one insulin/IGF-I receptor, whereas vertebrates have at least three. In mammals, although insulin is involved primarily in the control of glucose levels and metabolism and the primary role of IGF-I is to mediate the effects of GH on somatic growth, insulin and IGF-I, in addition to their respective receptors, exhibit extensive homology, functional overlap, cross talk, and similarity of intracellular signaling mechanisms.

Thus it is possible that a life span-regulatory function might be distributed among the different branches of this endocrine system. It is likely that different branches of the insulin/IGF-I-signaling network control different aspects of physiology and metabolism. Possibly, one branch of this network controls life span (35).

DISCLOSURES

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REFERENCES

1. Arai Y, Hirose N, Yamamura K, Shimizu K, Takayama M, Ebihara Y, and Osono Y. Serum insulin like growth factor 1(IGF-1) in centenarians: implication of IGF-1 as a turnover protein. *J Gerontol A Biol Sci Med Sci* 56A: M79-M82, 2001.
2. Arvat E, Broglio F, and Ghigo E. Insulin-like growth factor 1: implication in aging. *Drugs Aging* 16: 29-40, 2000.
3. Ashrafi K, Lin SS, Manchester JK, and Gordon JI. Sip2p and its partner Snf1p kinase affect aging in *S. cerevisiae*. *Genes Dev* 14: 1872-1885, 2000.
4. Bartke A. Mutation prolongs life in flies: implication for ageing in mammals. *Trends Endocrinol Metab* 12: 233-234, 2000.
5. Bartke A, Chandrashekar V, Dominici F, Turyn D, Kinney B, Steger R, and Kopchick JJ. Insulin-like growth factor 1 (IGF-1) and aging: controversies and new insights. *Biogerontology* 4: 1-8, 2003.
6. Blüher M, Kahn BB, and Kahn CR. Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* 299: 572-574, 2003.
7. Blüher M, Michael MD, Peroni OD, Ueki K, Carter N, Kahn BB, and Kahn CR. Adipose tissue selective insulin receptor knockout protects against obesity and obesity-related glucose intolerance. *Dev Cell* 3: 25-38, 2002.
8. Bonafè M, Barbieri M, Marchegiani F, Olivieri F, Ragno E, Giampieri C, Mugianesi E, Centurelli M, Franceschi C, and Paolisso G. Polymorphic variants of IGF-1R and phosphoinositide 3-kinase genes affect IGF-I plasma levels and human longevity: cues for an evolutionary-conserved mechanism of life-span control. *J Clin Endocrinol Metab* 88: 3299-3304, 2003.
9. Brown-Borg HM, Borg KF, Meliska CJ, and Bartke A. Dwarf mice and the ageing process. *Nature* 384: 33, 1996.
10. Carter CS, Ramsey MM, Ingram RL, Cashion AB, Cefalu WT, Wang ZQ, and Sonntag WE. Models of growth hormone and IGF-1 deficiency: application to studies of ageing process and life span determination. *J Gerontol A Biol Sci Med Sci* 57: B177-B188, 2002.
11. Charlton HM. Mouse mutants as models in endocrine research. *Q J Exp Physiol* 69: 655-676, 1984.
12. Clancy DJ, Gems D, Harshman LG, Oldham S, Stocker H, Hafen E, Leevers SJ, and Partridge L. Extension of life span by loss of CHICO, a *Drosophila* insulin receptor substrate protein. *Science* 292: 104-106, 2001.
13. Clemmons DR. Insulin like growth factor 1 and its binding proteins. In: *Endocrinology* (4th ed.), edited by de Groot LJ and Jamenson JL. Philadelphia, PA: WB Saunders, 2001, vol. 1, chapt. 33, p. 439-460.
14. Coschigano KT, Clemmons D, Bellush LL, and Kopchick JJ. Assessment of growth parameters and life span of GHR/BP gene-disrupted mice. *Endocrinology* 141: 2608-2613, 2000.
15. Cowen T. A heady message for life span regulation. *Trends Genet* 17: 109-112, 2001.
16. D'Costa AP, Ingram RL, Lenham JE, and Sonntag WE. The regulation and mechanisms of action of growth hormone and insulin-like growth factor 1 during normal ageing. *J Reprod Fertil Suppl* 46: 87-98, 1993.
17. DeFronzo RA and Ferrannini E. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular diseases. *Diabetes Care* 14: 173-194, 1991.
18. Dozmrov I, Bartke A, and Miller RA. Array-based expression analysis of mouse liver genes: effect of age and of the longevity mutant *Prop 1^{df}*. *J Gerontol A Biol Sci Med Sci* 56: B72-B80, 2000.
19. Dunn SE, Kari FW, French J, Leininger JR, Travlos G, Wilson R, and Barrett JC. Dietary restriction reduces insulin-like growth factor I levels, which modulates apoptosis, cell proliferation, and tumor progression in p53-deficient mice. *Cancer Res* 57: 4667-4672, 1997.
20. Fabrizio P, Liou LL, Moy VN, Diaspro A, Selverstone Valentine J, Gralla EB, and Longo VD. SOD2 functions downstream of Sch9 to extend longevity in yeast. *Genetics* 163: 35-46, 2003.
21. Fabrizio P, Pozza F, Pletcher SD, Gendron CM, and Longo VD. Regulation of longevity and stress resistance by Sch 9 in yeast. *Science* 292: 288-290, 2001.
22. Facchini FS, Hua N, Abbasi F, and Reaven GM. Insulin resistance as a predictor of age-related diseases. *J Clin Endocrinol Metab* 86: 3574-3578, 2001.
23. Finch CE and Ruvkun G. The genetics of aging. *Annu Rev Genomics Hum Genet* 2:435-462, 2001.

24. Flurkey K, Papaconstantinou J, Miller RA, and Harrison DE. Lifespan extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production. *Proc Natl Acad Sci USA* 98: 6736–6741, 2001.
25. Gems D and Partridge L. Insulin/IGF-1 signaling and ageing: seeing the bigger picture. *Curr Opin Genet Dev* 11: 287–292, 2001.
26. Hardie DG, Carling D, and Carlson M. The AMP-activated/SNF1 protein kinase subfamily: metabolic sensors of the eukaryotic cell? *Annu Rev Biochem* 67: 821–855, 1998.
27. Henderson ST and Johnson TE. daf-16 Integrates developmental and environmental inputs to mediate aging in the nematode *Caenorhabditis elegans*. *Curr Biol* 11: 1975–1980, 2001.
28. Holzenberger M, Dupont J, Ducos B, Leneuve P, Geloen A, Even PC, Cervera P, and Le Bouc Y. IGF-1 receptor regulates life span and resistance to oxidative stress in mice. *Nature* 421: 182–186, 2003.
29. Honda Y and Honda S. The daf-2 gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in *Caenorhabditis elegans*. *FASEB J* 13: 1385–1393, 1999.
30. Honda Y and Honda S. Oxidative stress and life span determination in the nematode *Caenorhabditis elegans*. *Ann NY Acad Sci* 959: 466–474, 2002.
31. Hsieh CC, de Ford JH, Flurkey K, Harrison DE, and Papaconstantinou J. Effects of the Pit1 mutation on the insulin signaling pathway: implication on the longevity of the long lived Snell dwarf mouse. *Mech Ageing Dev* 123: 1254–1255, 2002.
32. Kaiser U, Scharadt C, Brandscherdt D, Wollmer E, and Havemann K. Expression of insulin like growth factor receptors I and II in normal human breast and in lung cancer. *J Cancer Res Clin Oncol* 119: 665–668, 1993.
33. Kalant N, Stewart J, and Kaplan R. Effect of diet restriction on glucose metabolism and insulin responsiveness in aging rats. *Mech Ageing Dev* 46: 89–104, 1988.
34. Katz EB, Stenbit AE, Hatton K, DePinho R, and Charron MJ. Cardiac and adipose tissue abnormalities but not diabetes in mice deficient in GLUT4. *Nature* 377: 151–155, 1995.
35. Kenion C. A conserved regulatory system for ageing. *Cell* 105: 165–168, 2001.
36. Kimura KD, Tissenbaum HA, Liu Y, and Ruvkun G. Daf-2, an insulin receptor like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science* 277: 942–946, 1997.
37. Kirkwood TB. Evolution of ageing. *Mech Ageing Dev* 123: 737–745, 2002.
38. Li S, Crenshaw EB III, Rawson EJ, Simmons DM, Swanson LW, and Rosenfeld MG. Dwarf locus mutants lacking three pituitary cell types result from mutations in the POU-domain gene pit-1. *Nature* 347: 528–533, 1990.
39. Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E, Knowler WC, Bennett PH, and Bogardus C. Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus. Prospective studies of Pima Indians. *N Engl J Med* 329: 1988–1992, 1993.
40. Lin K, Dorman JB, Rodan A, and Kenyon C. Daf-16: an HNF-3/forhead family member that can function to double the life span of *Caenorhabditis elegans*. *Science* 278: 1319–1322, 1997.
41. Lin Su-Ju, Defossez PA, and Guarente L. Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. *Science* 289: 2126–2128, 2000.
42. Longo VD and Finch CE. Evolutionary medicine: from dwarf model systems to healthy centenarians? *Science* 299: 1342–1346, 2003.
43. Malone EA, Inoue T, and Thomas JH. Genetic analysis of the roles of daf-28 and age-1 in regulating *Caenorhabditis elegans* dauer formation. *Genetics* 143: 1193–1205, 1996.
44. Martinez-Pastor MT, Marchler G, Schuller C, Marchler-Bauer A, Ruis H, and Estruch F. The *Saccharomyces cerevisiae* zinc finger proteins Msn2p and Msn4p are required for transcriptional induction through the stress response element (STRE). *EMBO J* 15: 2227–2235, 1996.
45. Mecocci P, Polidori MC, Troiano L, Cherubini A, Cecchetti R, Pini G, Straatman M, Monti D, Stahl W, Sies H, Franceschi C, and Senin U. Plasma antioxidants and longevity: a study on healthy centenarians. *Free Radic Biol Med* 28: 1243–1248, 2000.
46. Migliaccio E, Giorgio M, Mele S, Pellicci G, Reboldi P, Pandolfi PP, Lanfranccone L, and Pellicci PG. The p66^{shc} adaptor protein controls oxidative stress response and life span in mammals. *Nature* 402: 309–312, 1999.
47. Monti D, Salvioli S, Capri M, Malorni W, Straface E, Cosarizza A, Botti B, Piacentini M, Baggio G, Barbi C, Valensin S, Bonafè M, and Franceschi C. Decreased susceptibility to oxidative stress-induced apoptosis of peripheral blood mononuclear cells from healthy elderly and centenarians. *Mech Ageing Dev* 121: 239–250, 2000.
48. Morris JZ, Tissenbaum HA, and Ruvkun G. A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in *Caenorhabditis elegans*. *Nature* 382: 536–539, 1996.
49. Muller F. Growth hormone receptor knockout (Laron) mice. Science's SAGE KE, tg 1. [Online] <http://sageke.sciencemag.org/cgi/content/full/sageke/2002/8/tg1> (27 February 2002).
50. Murakami S and Johnson TE. A genetic pathway conferring life extension and resistance to UV stress in *Caenorhabditis elegans*. *Genetics* 143: 1207, 1996.
51. Ogg S, Paradis S, Gottlieb S, Patterson GL, Lee L, Tissenbaum HA, and Ruvkun G. The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. *Nature* 389: 994–999, 1997.
52. Paolisso G, Ammendola S, Del Buono A, Gambardella A, Riordino M, Tagliamonte MR, Rizzo MR, Carella C, and Varricchio M. Serum levels of insulin-like growth factor-I (IGF-I) and IGF-binding protein-3 in healthy centenarians: relationship with plasma leptin and lipid concentration, insulin action and cognitive function. *J Clin Endocrinol Metab* 82: 2204–2209, 1997.
53. Paolisso G, Barbieri M, Bonafè M, and Franceschi C. Metabolic age remodeling: the lessons from centenarians. *Eur J Clin Invest* 30: 888–894, 2000.
54. Paolisso G, Barbieri M, Rizzo MR, Carella C, Rotondi M, Bonafè M, Franceschi G, Rose G, and De Benedictis G. Low insulin resistance and preserved β -cell function contribute to human longevity but are not associated with TH-INS genes. *Exp Gerontol* 37: 149–156, 2001.
55. Paolisso G, Gambardella A, Ammendola S, D'Amore A, and Varricchio M. Glucose tolerance and insulin action in healthy centenarians. *Am J Physiol Endocrinol Metab* 270: E890–E896, 1996.
56. Paolisso G, Tagliamonte MR, Rizzo MR, Manzella D, Gambardella A, and Varricchio M. Oxidative stress and advancing age: results in healthy centenarians. *J Am Geriatr Soc* 46: 833–838, 1998.
57. Patel MN, Knight CG, Karageorgi C, and Leroi AM. Evolution of germ-line signals that regulate growth and aging in nematodes. *Proc Natl Acad Sci USA* 99: 769–774, 2002.
58. Pierce SB, Costa M, Wisotzkey R, Devadhar S, Homburger SA, Buchman AR, Ferguson KC, Heller J, Platt DM, Pasquinelli AA, Liu LX, Doberstein SK, and Ruvkun G. Regulation of DAF-2 receptor signaling by human insulin and ins-1, a member of the unusually large and diverse *C. elegans* insulin gene family. *Genes Dev* 6: 672–686, 2001.
59. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 37: 1597–1606, 1988.
60. Ruan Y, Chen C, Cao Y, and Garofalo RS. The *Drosophila* insulin receptor contains a novel carboxyl-terminal extension likely to play an important role in signal transduction. *J Biol Chem* 270: 4236–4243, 1995.
61. Sonntag WE, Lenham JE, and Ingram RL. Effects of aging and dietary restriction on tissue protein synthesis: relationship to plasma insulin like growth factor-1. *J Gerontol A Biol Sci Med Sci* 47: B159–B163, 1992.
62. Sonntag WE, Lynch CD, Cefalu WT, Ingram RL, Bennet SA, Thornton P, and Khan AS. Pleiotropic effects of growth hormone and insulin-like growth factor (IGF)-1 on biological aging: inferences from moderate caloric restricted animals. *J Gerontol A Biol Sci Med Sci* 54: B521–B538, 1999.

63. Spiteller G. Lipid peroxidation in aging and age-dependent diseases. *Exp Gerontol* 36: 1425–1457, 2001.
64. Stadtman ER. Protein oxidation in aging and age-related diseases. *Ann NY Acad Sci* 928: 22–38, 2001.
65. Steger RW, Bartke A, and Cecim M. Premature ageing in transgenic mice expressing different growth hormone genes. *J Reprod Fertil Suppl* 46: 61–75, 1993.
66. Tatar M, Bartke A, and Antebi A. The endocrine regulation of aging by insulin-like signals. *Science* 299: 1346–1351, 2003.
67. Tatar M, Kopelman A, Epstein D, Tu MP, Yin CM, and Garofalo RS. A mutant *Drosophila* insulin receptor homolog that extends life span and impairs neuroendocrine function. *Science* 292: 107–110, 2000.
68. Taub J, Lau JF, Ma C, Hahn JH, Hoque R, Rothblatt J, and Chalfie M. A cytosolic catalase is needed to extend adult life span in *C. elegans* *daf-1* and *clk-1* mutants. *Nature* 399: 162–166, 1999.
69. Thatcher AR, Kannisto V, and Vaupel JW. The forces of mortality at age 80 to 120. In: *Odense Monographs on Population Aging*. Odense, Denmark: Odense Univ. Press, 1998, vol. 5.
70. Thevelein JM and de Winder JH. Novel sensing mechanisms and targets for the cAMP-protein kinase A pathway in the yeast *Saccharomyces cerevisiae*. *Mol Microbiol* 33: 904–918, 1999.
71. Tissenbaum HA and Ruvkun G. An insulin like signaling pathway affects both longevity and reproduction in *Caenorhabditis elegans*. *Genetics* 148: 703–718, 1998.
72. Vanfleteren JR and Braeckman BP. Mechanisms of life span determination in *Caenorhabditis elegans*. *Neurobiol Ageing* 20: 487–502, 1999.
73. Vanfleteren JR and De Vreese A. The gerontogenes age-1 and daf-2 determine metabolic rate potential in aging *Caenorhabditis elegans*. *FASEB J* 13: 1355–1361, 1995.
74. Wanagat J, Allison DB, and Weindruch R. Caloric intake and aging: mechanisms in rodents and a study in nonhuman primates. *Toxicol Sci* 52, Suppl 2: 35–40, 1999.
75. Wang YZ and Wong YC. Sex hormone induced prostatic carcinogenesis in the noble rat: the role of insulin like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF) in the development of prostate cancer. *Prostate* 35: 165–177, 1998.
76. Weindruch R and Sohal RS. Seminars in medicine of the Beth Israel Deaconess Medical Center. Caloric intake and aging. *N Engl J Med* 337: 986–994, 1997.
77. Weinkove D and Leivers S. The genetic control of organ growth: insight from *Drosophila*. *Curr Opin Genet Dev* 10: 75–80, 2000.
78. Wolkow CA, Kimura KD, Lee MS, and Ruvkun G. Regulation of *C. elegans* life-span by insulinlike signaling in the nervous system. *Science* 290: 147–150, 2000.
79. Yashin AI, De Benedictis G, Vaupel JW, Tan Q, Andreev KF, Iachine IA, Bonafè M, De Luca M, Valensin S, Carotenuto L, and Franceschi C. Genes, demography and life span: the contribution of demographic data and genetic studies on ageing and longevity. *Am J Hum Genet* 65: 1178–1193, 1999.

